Journal of Cellular Biochemistry 93:1134–1142 (2004)

Inhibition of Cell Proliferation in Human Breast Tumor Cells by Antisense Oligonucleotides Against Facilitative Glucose Transporter 5

K.K. Chan, Judy Y.W. Chan, Karen K.W. Chung, and Kwok-Pui Fung*

Department of Biochemistry, The Chinese University of Hong Kong, Hong Kong, China

Abstract In recent years, successful examples of antisense oligonucleotide (AS) therapy for genetic diseases have stimulated scientists to investigate its application on cancer diseases. AS can be used to down-regulate the mRNA and protein expression by annealing to specific region of the target mRNA which is responsible for the malignancy. Glucose transporter 5 (Glut5) is a tissue specific transporter that can be found on breast cancer tissues but not on normal breast tissues. Therefore, it is of clinical interest to investigate whether AS against Glut5 mRNA can tackle breast cancer. In this study, two cell lines, MCF-7 which is estrogen-receptor positive and MDA-MB-231 which is estrogen-receptor negative, were used to mimic breast cancer tissues at early and late stages, respectively. A 15-base sequence around the start codon of Glut5 was used. It was found that AS against Glut5 exerted anti-proliferative effect on both of these two breast tumor cell lines and seemed to exert its effect via the suppression of expression of Glut5 proteins in the cells. AS against Glut5 exhibited no effect on human hepatoma HepG2 cells which do not possess any Glut5. The results imply an alternative way in treating breast tumor as the AS against Glut5, unlike tamoxifen, takes effect on breast tumor cells via suppressing the expression of Glut5 that they specifically possess, and regardless whether the breast tumors are estrogen dependent or not. J. Cell. Biochem. 93: 1134–1142, 2004.

Key words: antisense; glucose transporter; breast tumor

Thirteen mammalian glucose transporters (Gluts) have been identified so far. They are named Glut1-12 and H(+)-myo-inositol cotransporter (HMIT), while Glut6 was found to be a pseudogene that cannot be detected in protein level [Wood and Trayhurn, 2003]. There is a close relationship between the Glut expression and malignant transformation. Some oncogenes can regulate glucose transport in tumor cells. In general, oncogene-transformed cells will exhibit an increase in glucose transport. It was found that *src*-transformed chicken embryo fibroblasts and *raf-1* transformed 3T3-L1 fibroblasts had an elevated glucose transport than the native one [White et al., 1991; Fingar and Birnbaum, 1994]. This change in glucose transport can be achieved by increasing the expression of a particular glucose transporter, decreasing the degradation rate of the transporter, or decreasing the Km through structural modification in Glut [Ahmed and Berridge, 1998]. Over-expression of a particular Glut was also common in various cancers. For example, Glut1 was found to be over-expressed in breast and colorectal carcinoma [Younes et al., 1995; Haber et al., 1998]. Other cancers such as hepatoma and ovarian carcinoma also over-express Glut2 and Glut3, respectively [Grobholz et al., 1993; Kurata et al., 1999]. Besides, high glucose utilization accompanied with a high proliferation rate could be observed in tumor cells. Due to their different substrate specificity and affinity, Gluts exhibit a tissue-specific localization. Among the family, Glut5, the most divergent member, was found to be a fructose transporter with a Km varied from 6 to 10 mM [Miyamoto et al., 1994]. It can only be located on the brush

Grant sponsor: Research Grants Council, Hong Kong, China (earmarked grant); Grant number: CUHK 4148/98M; Grant sponsor: Research Grants Council, Hong Kong, China (direct grant); Grant number: 2040955.

*Correspondence to: Kwok-Pui Fung, Department of Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong, China. E-mail: kpfung@cuhk.edu.hk

Received 28 July 2003; Accepted 17 November 2003

DOI 10.1002/jcb.20270

border membrane of small intestinal enterocytes and spermatozoa. Moreover, it is highly restricted to neoplastic mammary tissue rather than normal mammary epithelial cells [Zamora-Leon et al., 1996].

Antisense molecules are oligonucleotides whose sequences are complementary to RNA transcribed from the target gene. These molecules block gene expression by interacting with its RNA transcript [Agrawal and Zhao, 1998]. In principle, antisense oligonucleotides (AS) can be developed and applied against any gene product. The greatest impact of AS is anticipated in the area of cancer because effective treatments do not currently exist and because differences in genetic profile exist between diseased and healthy individuals [Kronenwett and Haas, 1998]. For examples, we [Chan et al., 2000; Chen et al., 2002] and other researchers [Noguchi et al., 2000] found that AS against Glut1 could suppress the growth of human tumor cells.

In this study, we tried to suppress the Glut5 expression on two breast tumor cell lines, MCF-7 and MDA-MB-231 cells, by using a 15-base phosphorothioated oligonucleotides. In this class of oligonucleotides, one of the oxygen atoms in the phosphate group is replaced with a sulfur atom. The resulting compound is negatively charged and much more resistant to nucleases [Agrawal and Zhao, 1998]. MCF-7 cells are estrogen-receptor positive and MDA-MB-231 cells are estrogen-receptor negative, mimicking breast cancer at early and late stages, respectively. Both MCF-7 and MDA-MB-231 cells showed a reduction in both Glut5 mRNA and protein levels after treatment. It is also accompanied with a decrease in cell proliferation rate.

METHODS

Cell Culture

Human tumor cell lines MCF-7, MDA-MB-231, and HepG2 were purchased from ATCC (Rockville, MD). They were grown in RPMI-1640 medium (Gibco BRL, Carlsbad, CA) supplemented with 10% fetal calf serum and cultured in a humidified atmosphere of 5% CO_2 at 37°C. Lipofectin was used as a carrier to enhance the uptake of the oligonucleotides. Lipofectin in the same amount (w/w 1:1) as the oligonucleotide was mixed together in serum free medium. After a 15 h transfection at 37°C, the serum-free medium was removed and replaced with fresh complete medium. The cells were further incubated at 37°C 5% CO_2 until another assay was carried out.

Design of Oligonucleotide Sequence

The sequence of AS against Glut5 was selected to be complementary to human Glut5 cDNA sequence around the start codon region started at base 70. For 15 base long AS against Glut5, the sequence was: 5′-TTG CTC CAT GCT TGC-3′. The sequence of sense control for Glut5 was: 5′-GCA AGC ATG GAG CAA-3′. All the oligonucleotides were phophorothioated at each base to increase their stability.

Cell Proliferation Assay

2×10^4 cells per well were seeded on 96-well plate. After 24 h incubation time, transfection was carried out [Chan et al., 2000]. At the appropriate time, the medium was removed and washed with PBS once. Volume of 50 μl of a 5 mg/ml solution of MTT was added into each well and the plate was incubated at 37°C for 2 h. Then, 150 μl of DMSO was added and incubated at room temperature for further 30 min. The optical density of each well was measured at 540 nm by an ELISA plate reader (BioRad, Hercules, CA).

Thymidine Incorporation

In a 24-well plate, 6×10^4 cells per well were seeded. After treating with 2 μM AS against Glut5, the medium was discarded and 0.5 μCi [methyl-^{3}H]-thymidine (Amersham Biosciences, Piscataway, NJ) in culture medium was added to each well. The plate was incubated at 37°C for 3 h. The medium was then removed and washed with PBS. One milliliter of 10% TCA was then added and incubated for 1 h at room temperature. TCA was discarded and followed by washing twice in PBS. Two hundred microliters of 0.1% SDS and 50 μl of 1 N NaOH were added to lyse the cells. The lysate was then measured for scintillation counting and its protein content was evaluated by BCA method.

RT-PCR Analysis

Four micromolar of AS against Glut5 was used to treat the tumor cells. TRIzol Reagent (Gibco BRL) was used to extract the total RNA after transfection and recovery. The RNA pellet was dried briefly and dissolved in DEPC-water. Three to four micrograms of the total RNA was used to synthesize the first strand cDNA by using the SuperScript Preamplification System (Gibco BRL). The forward primer sequence of

Glut5 was: 5′-TAG GGC AAG CTT CTG AAG TGT ACC CGG AAA AGG-3′ while the reverse primer sequence was: 5′-TAG GGC GCG GCC GCG AAA AGT GAT CAG GTT CAT-3′. These primers flanking a 700 base pairs product would amplify the Glut5 mRNA. For glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the forward primer sequence was: 5′-ACC ACA GTC CAT GCC ATC AC-3′ while the reverse primer sequence was: 5′-TCC ACC ACC CTG TTG CTG TA-3′. The amplified products were then electrophoresed on a 1% agarose gel and stained with ethidium bromide.

Western Hybridization

Four micromolar of AS against Glut5 was used to treat the tumor cells. Protein lysate was extracted at appropriate time point. The protein content of each sample was estimated by BCA protein assay. Twenty to twenty-five microgram samples were used to run SDS–PAGE (4.5% stacking gel and 8% running gel). The proteins were then transferred to a polyvinylidene fluoride (PVDF) microporous membrane by semi-dry transfer system (BioRad). The membrane was blocked in 10% non-fat milk (in TBS-T) before incubating with monoclonal rabbit antibody produced against human Glut5. After adding anti-rabbit IgG antibody conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA), ECL detection reagents (Amersham) were used and the emitting signal was detected by exposure to autoradiography film (Kodak, Rochester, NY). The bands were quantified by using densitometer (Molecular Dynamics, Amersham Biosciences).

Fructose Uptake Assay

Two micromolar of AS against Glut5 was used in transfection. At appropriate time, the medium was removed and the cells were washed with PBS. Fructose (0.1 mM) and 0.5 μCi ^{14}C-fructose for fructose uptake assay (Amersham) in PBS was added to each well for 15 min at 37°C. The PBS was discarded and washed with 10 mM fructose in normal saline. After that, 400 μl of 0.1% SDS was added. Volume of 10 μl was used for protein content determination and the rest was for scintillation counting.

Statistical Analysis

Data were expressed as the mean value ± SD in different experiments. Student's *t*-test was performed in the experiments. $P < 0.05$ indicated that the difference between the tested and control groups was statistically significant.

RESULTS

RT-PCR Analysis

The total RNA were extracted from both cell lines after treated with AS against Glut5 and the Glut5 mRNA level was measured by using RT-PCR. As observed in Figure 1A, the level of Glut5 mRNA in MCF-7 cells was suppressed by 77.5% at 5 h after transfection when compared with the level in serum-free control. At 48 h post-transfection, the effect of the AS against Glut5 was still valid by which the suppression was found to be 46.5%. For MDA-MB-231 cells, suppressions of Glut5 mRNA were also observed at 5 and 24 h after the transfection, with the suppression of 88.8 and 80.7%, comparing with serum-free controls, respectively (Fig. 1B). When comparing with the level of Glut5 mRNA in antisense treated tumor cells, the degree of suppression is highest at 24 h after transfection in MCF-7 and MDA-MB-231 tumor cells (Fig. 2).

Western Analysis

The probable change in Glut5 protein expression in MCF-7 and MDA-MB-231 cells after transfection of AS against Glut5 were measured by Western hybridization. The results showed that after recovery from transfection for 1 day, the amount of Glut5 proteins in AS treated cells were lower than the sense (S) control (Fig. 3). Combined with the results in RT-PCR, the reduction of Glut5 mRNA by AS of Glut5 had subsequently led to suppression of translation of Glut5 mRNA to Glut5 protein. This suppression was shown in both MCF-7 and MDA-MB-231 cells. After 24 h of transfection, in MCF-7 cells, the Glut5 protein amounts were about 4% less than the sense control while about 20% reduction was detected in MDA-MB-231 cells. The suppression of translation could also be observed at 48 h after transfection. In MCF-7 cells, the suppression of Glut5 protein by AS of Glut5 was found to be about 36% comparing with sense control. On the other hand, the Glut5 protein level was about 18% less in the antisense treated MDA-MB-231cells when compared with sense control.

Cell Proliferation Assay

The effect of the AS against Glut5 on the proliferation rate of both cell lines was investigated

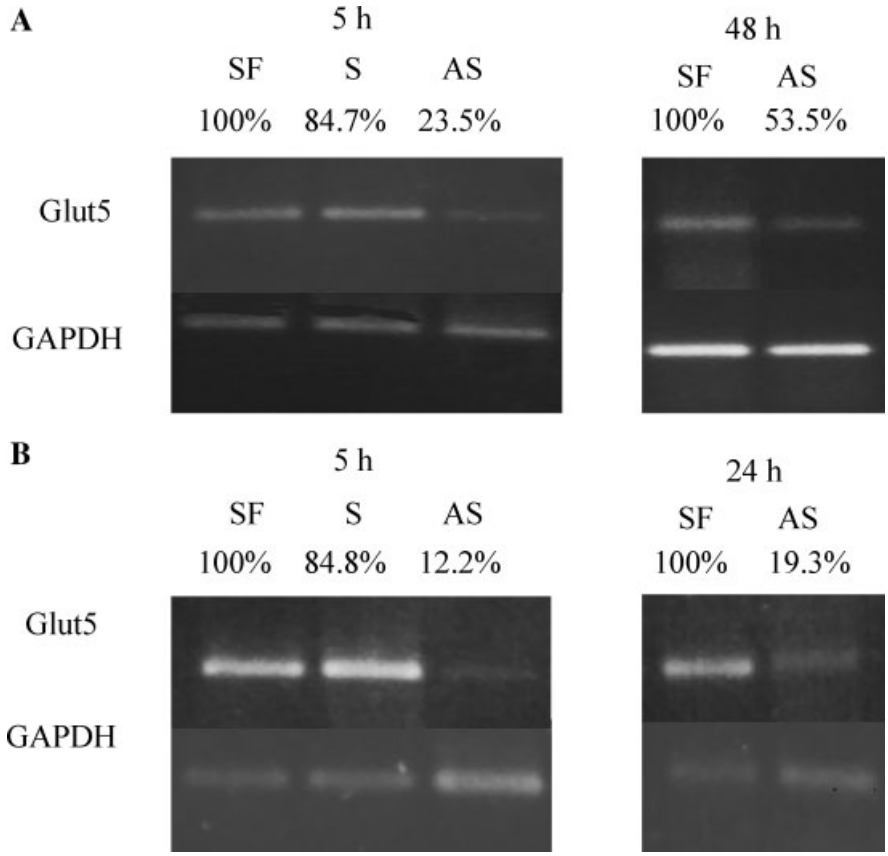

Fig. 1. The suppression of Glut5 mRNA in (**A**) MCF-7 and (**B**) MDA-MB-231 cells after treated with antisense oligonucleotide (AS) against Glut5. Total RNA was extracted from cells treated with serum-free medium (SF), sense sequence (S), and AS against Glut5, respectively, at the time point indicated after transfection. The RNA was then reverse transcribed into cDNA which would carried out PCR by specific primers. The amounts of samples added were normalized by amplification of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The PCR products were then run in 1% agarose gel and stained with ethidium bromide. The intensity of the bands were analyzed by densitometer. The experiments were repeated twice. Only one of the results was shown here. Not more than 10% in SD were obtained in the two experiments.

by using MTT assay. For the 15-base Glut5 AS, anti-proliferative effect was observed in both MCF-7 and MDA-MB-231 cells (Fig. 4). The anti-proliferative effect exhibited a concentration-dependent manner. The non-specific effect was relative low as indicated by a higher percentage of survival in sense (S) treatment. In addition, the anti-proliferative effect of the AS was similar in both cell lines. Similar percentage was observed at the same concentration of AS.

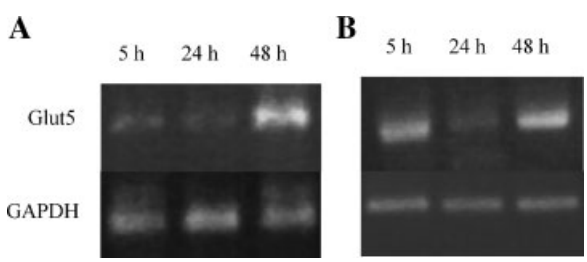

Fig. 2. Comparison of the Glut5 mRNA in MCF-7 and MDA-MB-231 cells treated with AS against Glut5 at different time points. **A**, MCF-7 cells; **B**, MDA-MB-231 cells.

To verify the specificity of anti-Glut5 oligonucleotide on cells that expressed Glut5 only, the AS against Glut5 was applied to HepG2 cells. HepG2 cell line is a hepatocarcinoma cell line that expresses Glut1, Glut2, and Glut3 but no Glut5 expression was detected [Yamamoto et al., 1990]. Therefore, HepG2 cell line is a good indicator for the specificity of the effect of AS against Glut5 in the present study. From the results on Figure 4C, the anti-proliferative effect of the AS against Glut5 on MDA-MB-231 cells was significantly higher than that on HepG2 cells. The difference was found to be significant ($P < 0.05$) and the level of antisense

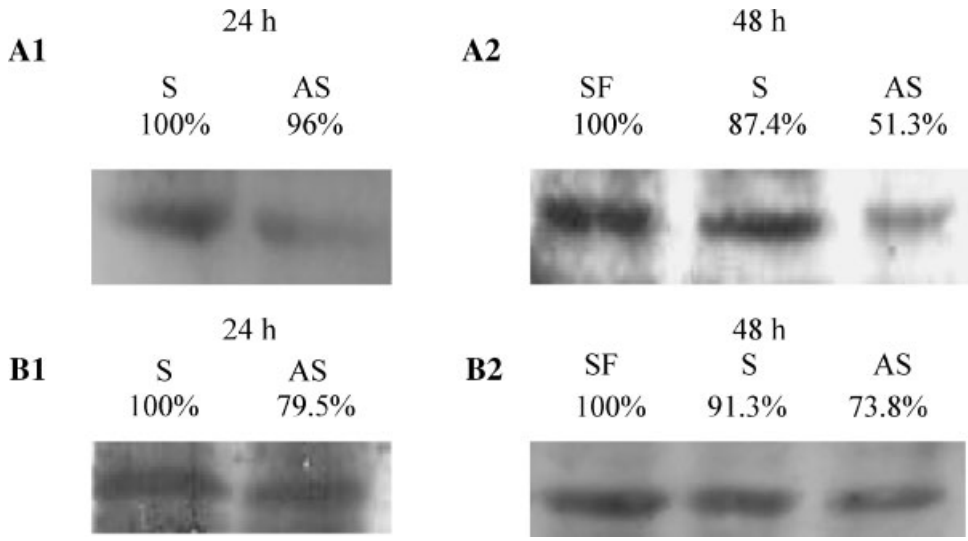

Fig. 3. The suppression of Glut5 in protein levels in MCF-7 and MDA-MB-231 cell lines after treatment of AS against Glut5. At 24 h (1) and 48 h (2) after transfection, cells with different treatments were trypsinized and the proteins were extracted as described in ''Methods.'' The protein samples were then electrophoresed in SDS–PAGE. The Glut5 proteins were blotted against anti-rabbit Glut5 antibodies and developed by ECL method. The intensities of the bands were analyzed by densitometer. **A**, MCF-7 cells; **B**, MDA-MB-231cells; SF, serum-free medium; S, sense oligonucleotide; AS, antisense oligonucleotide. The experiments were repeated twice. Only one of the results was shown here. Not more than 10% in SD were obtained in the two experiments.

effect on HepG2 cells was similar with the non-specific effect caused by the sense oligonucleotides. This result indicated that AS against Glut5 was more effective on tumor cells with Glut5 expression, i.e., MCF-7 and MDA-MB-231 cells.

Thymidine Incorporation

To confirm that the growth of the tumor cells was inhibited, the amount of DNA synthesis during S phase can be detected by monitoring the incorporation of radioactive DNA precursors [methyl-^{3}H]-thymidine. From the results on Figure 5, the counts measured in AS against Glut5 treated MCF-7 and MDA-MB-231 cells were lower than both serum-free and sense controls. This anti-proliferative effect could also be observed until 48 h after transfection while the differences of thymidine incorporated on both cell lines were enhanced than 24 h recovery.

Fructose Uptake Measurement

Carbon-14 labeled fructose was used to follow the change in cellular uptake. For MCF-7 cells, the fructose uptake was reduced by the AS against Glut5 either at 24 or 48 h after transfection (Fig. 6A). The amount of fructose uptake was significantly lower when compared with the sense control. For MDA-MB-231 cells, similar results were observed.

DISCUSSION

AS is a short stretch of synthetic, chemically modified nucleic acids. High specificity of antisense mechanism can even distinguish sequences difference between one base [Mercatante and Kole, 2000]. Moreover, the suppressive effect can occur at the level of both mRNA and protein. These characteristics make AS be a potential agent on many therapies [Giovine et al., 1998; Miayake et al., 2000].

In our study, we have tried to investigate the effect of AS against Glut5 on breast tumor cells, basing on the findings that alteration of glucose transporter-mediated glucose transport would affect the growth of tumor cells [Fung et al., 1986; Noguchi et al., 2000; Chen et al., 2002]. Glut5, a breast tumor specific glucose transporter, was chosen to be the target because the normal breast tissues do not express Glut5 [Zamora-Leon et al., 1996]. A previous research found that suppression of Glut1 mRNA could suppress the growth of a gastric cancer cell line [Noguchi et al., 2000]. We [Chan et al., 2000; Chen et al., 2002] also found that AS against Glut1 could suppress the growth of human hepatoma HepG2 cell line. It was speculated that upset of the Glut5 mediated glucose transport would also inhibit the growth of breast tumor cells.

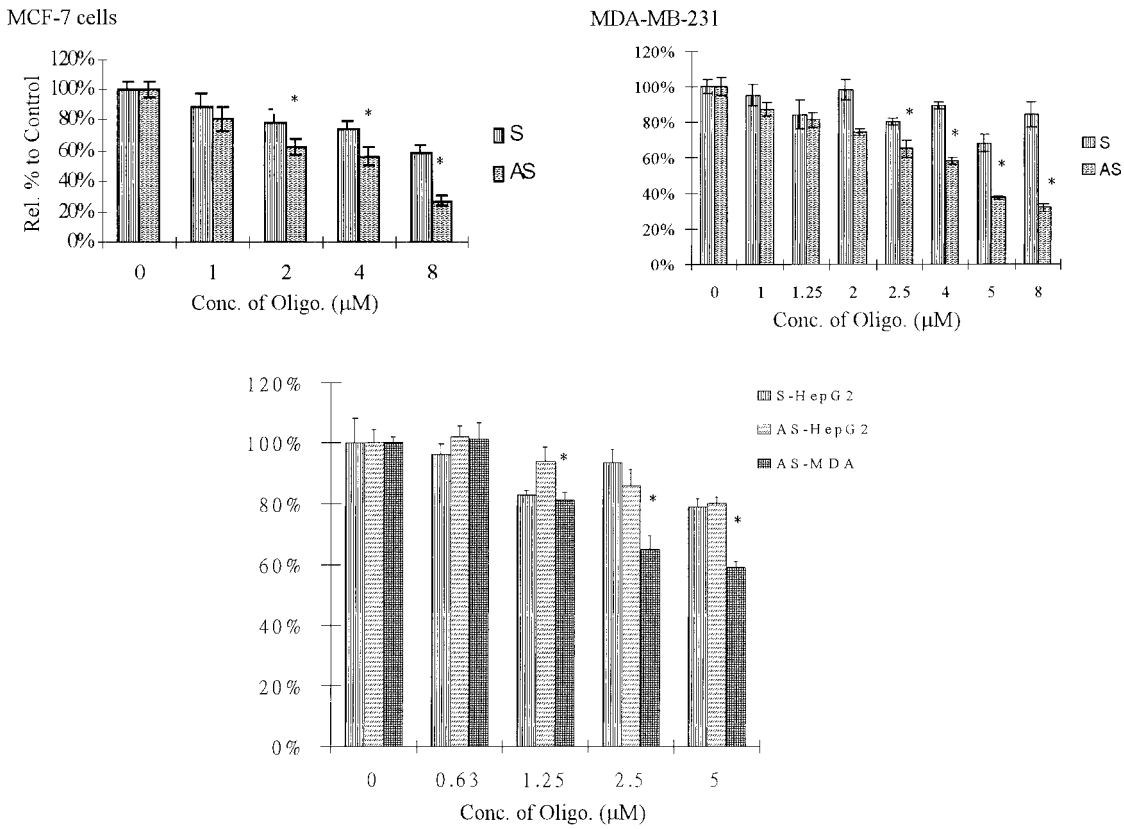

Fig. 4. The effect of AS against Glut5 on the growth of MCF-7 and MDA-MB-231 cells. Different concentrations of oligonucleotide as indicated was added to 2×10^4 cells/well in 96-well plate. Equal amount of lipofectin (w/w) was used to complex with the oligonucleotide in serum-free medium. After 15 h transfection, the cells were recovered in fresh complete medium for further 48 h. The percentage of cell confluence relative to serum-free treated control was measured by MTT assay. Sense sequence (S) was also added to differentiate any non-specific effect observed in antisense treatment (AS). Each bar represented the mean value of five replicates ±SD. HepG2 cells were treated as the same conditions as MDA-MB-231 cells. S-HepG2: HepG2 cells treated with Glut5 sense oligonucleotides; AS-HepG2: HepG2 cells treated with Glut5 antisense oligonucleotides; AS-MDA: MDA-MB-231 cells treated with Glut5 antisense oligonucleoides. $^*P < 0.05$.

By RT-PCR, the amount of mRNA in AS against Glut5 treated MCF-7 and MDA-MB-231 cells is less than the serum-free and sense control. From our results, the suppression of Glut5 mRNA could be observed as soon as 5 h after transfection. The reduction of mRNA could only be observed in antisense treatment (Figs. 1 and 2). This illustrated that only AS against Glut5 bound to target RNA specifically but not the sense oligonucleotides. The suppression of Glut5 mRNA lasted for 48 h after transfection. The level of mRNA recovered back to normal level as the serum-free and sense controls for 24 h further, i.e., at 96 h after transfection (data not shown). Moreover, the trend of changes in mRNA level was similar in both tumor cell lines. The suppression was most pronounced at 24 h after the transfection. The level of Glut5 mRNA then increased gradually. This may be due to the beginning of degradation of the AS inside the cells. Single dose of AS against Glut5 could last for about 48 h.

The suppression of mRNA of Glut5 by AS against Glut5 should inevitably lead to decline in the corresponding protein expression. Through the changes in protein level, the phenotypes or physiological conditions of cells will be affected. The amount of Glut5 proteins was less in AS against Glut5 treated cells than the amount in sense control (Fig. 3). The suppression of Glut5 proteins could be observed at 24 and 48 h after the transfection. In MCF-7 cells, the degree of suppression of Glut5 protein was less than the suppression in MDA-MB-231 cells at 24 h after transfection. However, as the recovery period kept going, this subtle inhibition of protein synthesis in MCF-7 cells was changed to be more pronounced at 48 h. The

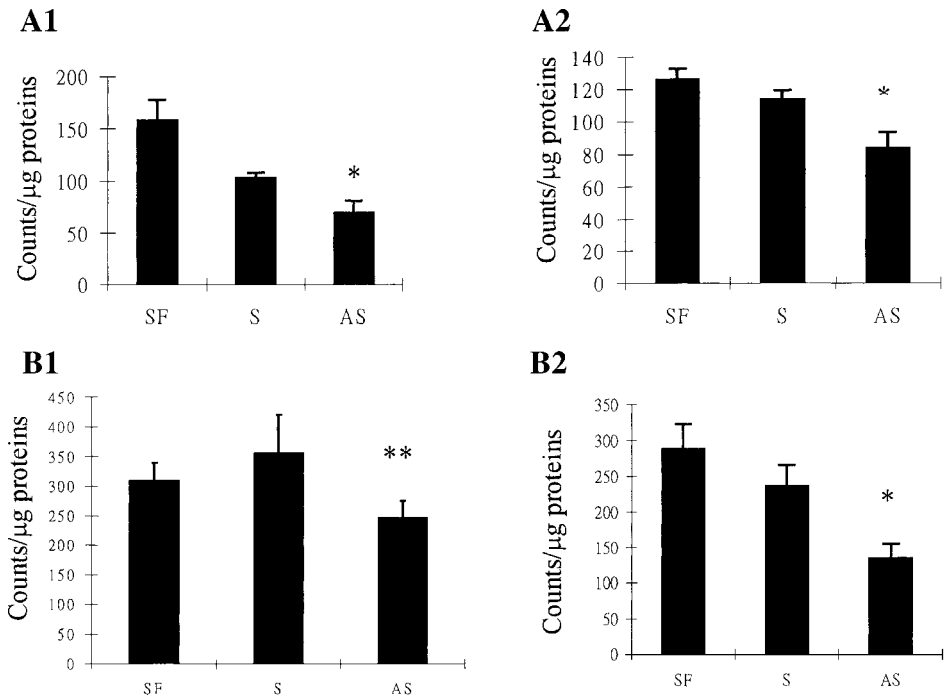

Fig. 5. The anti-proliferative effect of AS against Glut5 on MCF-7 and MDA-MB-231 cells. After 24 h (1) or 48 h (2) recovery from transfection, [methyl-^{3}H]-thymidine in 0.5 μCi was added to cells for further incubation for 3 h. The amount of thymidine uptake was measured by scintillation counting. Five microliters of the cell lysate was used for measuring of the protein amounts. Data were expressed as the mean value of triplicate experiments ±SD (**A**) MCF-7; (**B**) MDA-MB-231. The significance of the differences were analyzed by Student's *t*-test; $^*P < 0.001$, $^{**}P < 0.02$.

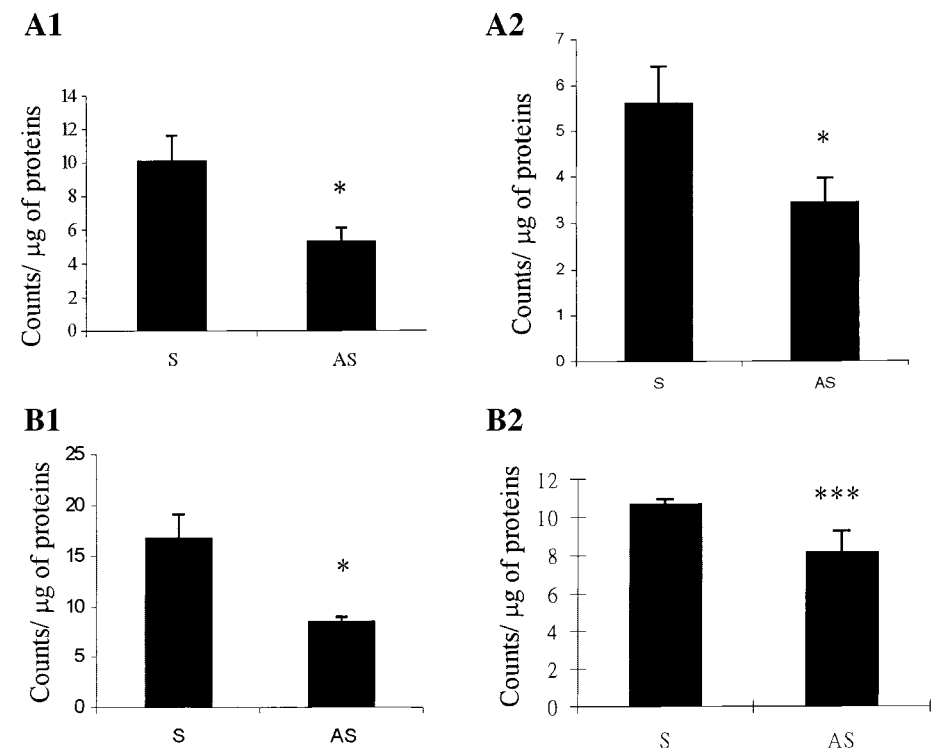

Fig. 6. The effect of AS against Glut5 on the fructose uptake of MCF-7 and MDA-MB-231 cells. The amount of fructose uptake was measured in antisense treated cells and the sense-sequence treated cells after (1) 24 h and (2) 48 h after transfection. **A**, MCF-7 cells; **B**, MDA-MB-231 cells. Data were expressed as the mean value of four replicates ±SD. The significance of the differences were analyzed by Student's *t*-test. S, sense oligonucleotide; AS, antisense oligonucleotide. $^*P < 0.001$; $^{***}P < 0.03$.

inhibitory effect transited from mRNA to protein level seemed to be slower in MCF-7 cells than that in MDA-MB-231cells.

After applying AS against Glut5, both breast tumor cell lines showed a reduction in the growth rate. The MTT assay indicated a dose-dependent response to the AS against Glut5 (Fig. 4). By [methyl-^{3}H]-thymidine incorporation assay, the amounts of DNA synthesis in both MCF-7 and MDA-MB-231 cells were found to be significantly suppressed in AS against Glut5 treatment than that in sense oligonucleotide treatment after 24 and 48 h (Fig. 5). Moreover, the antisense effect was highly specific on both breast tumor cells with Glut5 expression only (Fig. 5). For those tumor cells without Glut5, such as HepG2 cells, only non-specific effect can be obtained. The high specificity of using this AS against Glut5 may reduce any side effects on other tissues when it is applied clinically, because most of them (including normal breast cells) do not possess Glut5. This is one of the major reasons why we focus on Glut5 rather than other glucose transporters.

Besides the anti-proliferative effect, some of the physiological changes have been investigated. As expected, the fructose uptake in antisense treated tumor cells was found to be reduced to a greater extent in AS against Glut5 treated breast tumor cells. It is consistent with the results showing an inhibition on Glut5 translation. The decrease in fructose uptake could be attained during the period of time in which the expression of Glut5 mRNA and then proteins were suppressed (Fig. 6). Moreover, it seems that there is no up-regulation of other glucose transporters because there is no compensatory increase in glucose uptake after fructose uptake is suppressed (data not shown).

In conclusion, AS against Glut5 can exert anti-proliferative effect on Glut5-containing breast tumor cells but not for tumor cells without Glut5 expression such as HepG2 cells. In addition, AS against Glut5 can take effect on both MCF-7 cells which are estrogen-receptor positive and MDA-MB-231 cells which are estrogen-receptor negative. It is unlike tamoxifen, a common drug for treating breast cancer, which is not effective on estrogen-receptor negative cells, e.g., MDA-MB-231 cells used in the present study. As a result, estrogen-receptor negative cells in the breast cancer patient continue to grow and the breast cancer at the later stage of the patient cannot be treated by tamoxifen. The feasibility test of AS against Glut 5 treatment in vivo in nude mice bearing MCF-7 or MDA-MB-231 cells are in progress.

REFERENCES

Agrawal S, Zhao Q. 1998. Antisense therapeutics. Curr Opin Chem Biol 2:519–528.

Ahmed N, Berridge MV. 1998. Transforming oncogenes regulate glucose transport by increasing transporter affinity for glucose: Contrasting effects of oncogenes and heat stress in a murine marrow-derived cell line. Life Sci 63:1887–1903.

Chan JY, Chu AC, Fung KP. 2000. Inhibition of P-glycoprotein expression and reversal of drug resistance of human hepatoma HepG2 cells by multidrug resistance gene (mdr1) antisense RNA. Life Sci 67:2117–2124.

Chen CP, Li XX, Zhang LR, Min JM, Chan JY, Fung KP, Wang SQ, Zhang LH. 2002. Synthesis of antisense oligonucleotide-peptide conjugate targeting to GLUT-1 in HepG-2 and MCF-7 Cells. Bioconjug Chem 13:525–529.

Fingar DC, Birnbaum MJ. 1994. A role for Raf-1 in the divergent signaling pathways mediating insulin-stimulated glucose transport. J Biol Chem 269:10127–10132.

Fung KP, Choy YM, Chan TW, Lam WP, Lee CY. 1986. Glucose regulates its own transport in Ehrlich ascites tumour cells. Biochem Biophys Res Commun 134:1231–1237.

Giovine M, Gasparini A, Scarfi S, Damonte G, Sturla L, Millo E, Tonetti M, Benatti U. 1998. Synthesis and characterization of a specific peptide nucleic acid that inhibits expression of inducible NO synthase. FEBS Lett 426:33–36.

Grobholz R, Hacker HJ, Thorens B, Bannasch P. 1993. Reduction in the expression of glucose transporter protein GLUT 2 in preneoplastic and neoplastic hepatic lesions and re-expression of GLUT 1 in late stages of hepatocarcinogenesis. Cancer Res 53:4204–4211.

Haber RS, Rathan A, Weiser KR, Pritsker A, Itzkowitz SH, Bodian C, Slater G, Weiss A, Burstein DE. 1998. GLUT1 glucose transporter expression in colorectal carcinoma: A marker for poor prognosis. Cancer 83:34–40.

Kronenwett R, Haas R. 1998. Antisense strategies for the treatment of hematological malignancies and solid tumors. Ann Hematol 77:1–12.

Kurata T, Oguri T, Isobe T, Ishioka S, Yamakido M. 1999. Differential expression of facilitative glucose transporter (*GLUT*) genes in primary lung cancers and their liver metastases. Jpn J Cancer Res 90:1238–1243.

Mercatante D, Kole R. 2000. Modification of alternative splicing pathways as a potential approach to chemotherapy. Pharmacol Ther 85:237–243.

Miayake H, Tolcher A, Gleave ME. 2000. Chemosensitizatioon and delayed androgen-independent recurrence of prostate cancer with the use of antisense Bcl-2 oligonucleotides. J Natl Cancer Inst 92:34–41.

Miyamoto K, Tatsumi S, Morimoto A, Minami H, Yamamoto H, Sone K, Taketani Y, Nakabou Y, Oka T, Takeda E. 1994. Characterization of the rabbit intestinal fructose transporter (GLUT5). Biochem J 303:877–883.

Noguchi Y, Saito A, Miyagi Y, Yamanaka S, Marat D, Doi C, Yoshikawa T, Tsuburaya A, Ito T, Satoh S. 2000.

Suppression of facilitative glucose transporter 1 mRNA can suppress tumor growth. Cancer Lett 154:175–182.
White MK, Rall TB, Weber MJ. 1991. Differential regulation of glucose transporter isoforms by the src oncogene in chicken embryo fibroblasts. Mol Cell Biol 11:4448–4454.
Wood IS, Trayhurn P. 2003. Glucose transporters (GLUT and SGLT): Expanded families of sugar transport proteins. Br J Nutr 89:3–9.
Yamamoto T, Seino Y, Fukumoto H, Koh G, Yano H, Inagaki N, Yamada Y, Inoue K, Manabe T, Imura H. 1990. Over-expression of facilitative glucose transporter genes in human cancer. Biochem Biophys Res Commun 170:223–230.
Younes M, Brown RW, Mody DR, Fernandez L, Laucirica R. 1995. GLUT1 expression in human breast carcinoma: Correlation with known prognostic markers. Anticancer Res 15:2895–2898.
Zamora-Leon SP, Golde DW, Concha II, Rivas CI, Delgado-Lopez F, Baselga J, Nualart F, Vera JC. 1996. Expression of the fructose transporter GLUT5 in human breast cancer. Proc Natl Acad Sci 93:1847–1852.